IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

TOIVOLA et al Serial

No. 497,813

filed 25th May, 1983

for

"NOVEL TRI-PHENYL ALKANE AND ALKENE DERIVATIVES AND THEIR PREPARATION AND USE"

D E C L A R A T I O N

I, LAURI SAKARI NIEMINEN, declare:

1. That I am a citizen of Finland of Valppakuja 4, 21360 Lieto AS, Finland. I am a ~~Doctor~~ Docent of Toxicology at the University of Jyvaskyla, Finland and a Doctor of Philosophy. The following experiments have been carried out under my supervision to compare the subacute toxicity of the known drug tamoxifen with that of a compound supplied to me by Farmos Group Ltd. and identified by them as 4-chloro-1,2-diphenyl-1-[4-[2-(N,N-dimethylamino) ethoxy]phenyl]-1-butene, herein after called compound 7. 31/10-84 (LN)

2. Under my supervision, the toxicities of compound 7 and tamoxifen citrate were compared in female rats in a toxicity test as follows. The oral dose levels used were 10 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg. The dosing took place seven days a week for a three week period. There were 5 or 6 female rats in each group.

The following mortalities were observed:

tamoxifen	10 mg/kg	0/5
"	50 mg/kg	0/5
"	100 mg/kg	6/6
"	150 mg/kg	5/5
"	200 mg/kg	6/6
compound 7	10 mg/kg	0/5
"	50 mg/kg	0/5
"	100 mg/kg	0/6
"	150 mg/kg	5/5
"	200 mg/kg	6/6

The deaths caused by tamoxifen happened in the end of the first week of testing and during the second week. With one exception, the deaths caused by compound 7 did not happen until the second and the third weeks of testing.

3. The autopsies performed indicated that the cause of death both with tamoxifen and compound 7 was acute gastric dilatation. This fatal disease condition is probably caused by the atony of the stomach. The changed hormonal balance could be one possible reason for the development of the disease condition. No macroscopic organ damage caused by the drug was observed in the autopsies performed on the dead and the surviving animals. This comparative toxicity test showed that tamoxifen citrate is more toxic in female rats than compound 7.

On the basis of the performed autopsies it seems evident that both substances cause the death of the animals with the same mechanism.

4. The oral subacute toxicities of compound 7 citrate and tamoxifen citrate were studied also in male rats. The dose levels used were 10 mg/kg, 50 mg/kg, and 100 mg/kg. The dosing took place seven days a week over a threeweek period. Each group consisted of 4 or 5 male rats. No deaths were observed during the test. All dose levels of both substances caused a slight inhibition of the weight development of the same magnitude in both cases. No dose levels of compound 7 caused any changes in behaviour and appearance. However, in the largest tamoxifen group bloodiness was observed around the mouth and in the front legs and also fur loss was seen. This indicates a greater toxic effect of tamoxifen.

5. The 4 and 24 weeks' toxicities of compound 7 citrate were studied in rats. The oral dose levels used ~~were 3 mg/kg, 12 mg/kg, and~~ 48 mg/kg. Each dose group and the control group were made up of 15 rats of both sexes. The dosing was daily, peroral, seven days a week. After four weeks of dosing, 5 animals per sex were killed from each group. The animals to be killed had been chosen before the beginning of the test. The dosing continued for the rest of the animals (10 per sex per group) and they were killed after 24 weeks of dosing. It was observed in the study that tamoxifen caused greater loss of fur than compound 7. In addition, occasional slight bloodiness in the nostrils was observed in the animals of the tamoxifen group. This symptom is induced by the acute gastric dilatation that tamoxifen causes at

smaller dose levels than compound 7. No mortalities caused by compound 7 were observed in the test. On the contrary, the one death in the tamoxifen group could be connected with the drug. Both drugs were observed to cause equal inhibition of weight development that might be due to lessened food consumption.

6. The atrophying effect of tamoxifen and compound 7 on the sex organs of the female rats seemed equal. On the other hand, the atrophying effet of tamoxifen seemed greater on the sex organs of the male rats. In the 24 weeks' study tamoxifen induced ~~fatal~~ liver cell proliferation (nodular hyperplasia) at the 48 mg/kg dose level in all rats. This phenomenon was not seen in animals which received compound 7. 21/10-87 LW.

7. I conclude from these results that compound 7 has a significantly lower toxicity than tamoxifen. It is especially remarkable that in the first test the 6 female rats died after a dose of 100 mg/kg of tamoxifen whereas there was no mortality in the group that got the same dose of compound 7. This difference is particularly significant because it is necessary to use high dosages of these compounds if any useful effect against uterus sarcoma is to be obtained. I conclude that compound 7 is a superior antitumour agent to tamoxifen since the ratio between the effective dose and the toxic dose appears to be significantly more favourable than in the case of tamoxifen.

8. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; further that these statements are made with the knowledge that wilful

false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the Application or any Patent issuing thereon.

LAURI SAKARI NIEMINEN

Dated this 31 day of October 1984